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Fee 170:-

NEW RECEPTORSFIELD OF THE INVENTION

5 The present invention relates to novel vitamin D receptor related (VDRR) poly-peptides. Nucleic acid sequences encoding the same, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention, and uses thereof.

10

BACKGROUND OF THE INVENTION

Nuclear hormone receptors is a large group of conditionally regulated transcription factors. These receptors are activated and regulate target gene expression in response to 15 binding a variety of small chemical molecules (ligands) including steroids, vitamin D3, retinoids, eicosanoides (prostanoids), thyroid hormone and cholesterol derivatives.

A growing number of structurally related receptors have been identified for which no ligands yet have been identified. This group of receptors are referred to as orphan nuclear receptors (ONRs). A review of the ONRs can be found in Enmark et al, Mol. Endo., vol. 10, 20 No. 11 (1996) pp. 1293-1307, which is hereby incorporated by reference. The pivotal importance of a number of ONRs for processes such as metabolic homeostasis, cell differentiation and development have been demonstrated both by biochemical and genetic techniques. In addition, several ONRs have also been implicated as key factors in a variety of common diseases and disorders such as diabetes, obesity, inflammatory conditions and 25 proliferative diseases.

Based on these findings it is generally believed that novel ONRs are going to become potential drug targets for therapeutic invention of common diseases. Thus, it is of great importance to identify such receptors.

SUMMARY OF THE INVENTION

The present invention relates to novel vitamin D receptor related (VDRR) polypeptides, and formulations containing the same. Nucleic acid sequences encoding the VDRR polypeptides, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention. The invention further relates to VDRR polypeptides for use as medicaments, and use of substances affecting VDRR signal transduction for the manufacture of medicaments for treating metabolic, proliferative or inflammatory conditions. The present invention also relates to methods for identifying clones encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods for identifying substances for treatment of conditions affected by a VDRR polypeptide. More specifically, the novel VDRR polypeptide can be the polypeptide designated VDRR γ , which may be regulated by any small chemical molecule similar in structure to known ligands for nuclear receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 - The cDNA sequence encoding the novel nuclear receptor polypeptide vitamin D receptor related gamma (VDRR γ) is shown.

Figure 2 - Evolutionary neighbor-joining tree for VDRR γ as given by DBD-HMM alignment.

Figure 3 - Evolutionary neighbor-joining tree for VDRR γ as given by LBD-HMM alignment.

Figure 4 - The deduced amino acid sequence of VDRR γ is shown.

Figure 5 - Expression of VDRR γ in adult human tissues. The numbers on the right hand side, refer to kilobasepairs of the mRNA.

Figure 6 - Vitamin D3 transactivate a GAL4-DBD/VDR-LBD fusion protein but not a GAL4-DBD/VDRR γ -LBD fusion protein in transient transfections of CV-1 cells. The number on the left hand side refer to relative luciferase activity of the GAL4-luciferase reporter gene.

DETAILED DESCRIPTION OF THE INVENTION

The objects above are met by the present invention, which relates to an isolated or
5 recombinant nucleic acid comprising a contiguous nucleic acid sequence encoding a vitamin
D receptor related (VDRR) polypeptide. The VDRR polypeptide is suitably of mammalian,
preferably human, origin.

In preferred embodiments of the present invention, the nucleic acid encoding the
VDRR polypeptide contains a DNA-binding domain (DBD) comprising about 77 amino
10 acids with 9 cysteine residues. The DBD is further characterized by the following amino
acid sequence identity relative to the DBDs of human Vitamin D Receptor (hVDR) and
Orphan Nuclear Receptor 1 isolated from *Xenopus laevis* (xONR1), respectively:
(i) at least about 50% amino acid sequence identity with the DBD of hVDR, suitably at least
60% amino acid sequence identity with the DBD of hVDR; and
15 (ii) at least about 55% amino acid sequence identity with the DBD of xONR1, suitably
at least 65% amino acid sequence identity with the DBD of xONR1.
More particularly, the amino acid sequence identity relative to the DBDs of hVDR and
xONR1, respectively is
(i) about 65% amino acid sequence identity with the DBD of hVDR; and
20 (ii) about 71% amino acid sequence identity with the DBD of xONR1.

In preferred embodiments of the present invention, the nucleic acid encoding the
VDRR polypeptide contains a ligand-binding domain (LBD) characterized by the following
amino acid sequence identity, relative to the LBDs of hVDR and xONR1,
respectively:
25 (i) at least about 30% amino acid sequence identity with the LBD of hVDR, suitably at least
35% amino acid sequence identity with the LBD of hVDR; and
(ii) at least about 40% amino acid sequence identity with the LBD of xONR1, suitably
at least 45% amino acid sequence identity with the LBD of xONR1.
More particularly, the amino acid sequence identity relative to the LBDs of hVDR and
30 xONR1, respectively is
(i) about 42% amino acid sequence identity with the LBD of hVDR; and

(ii) about 54% amino acid sequence identity with the LBD of xONR1.

In a particularly preferred, the nucleic acid sequence of the present invention is that given in Fig. 1.

The present invention also relates to a nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR polypeptide in a sample. Suitably, the probe comprises at least 14 contiguous nucleotides, and preferably at least 28 contiguous nucleotides, of the nucleic acid sequence given in Fig. 1. The nucleic acid probe can be used in a method for identifying clones encoding a VDRR polypeptide, wherein the method comprises screening a genomic or cDNA library with the probe under low stringency hybridization conditions, and identifying those clones which display a substantial degree of hybridization to said probe.

The present invention further relates to an isolated or recombinant VDRR polypeptide. The polypeptide can be full-length, at which the sequence of amino acids is identical to the corresponding sequence found in mammals in general, and in human beings in particular. In the present invention, the polypeptide can also be a truncated, extended or mutated form of the full-length polypeptide. Truncated and extended forms relate to VDRR polypeptides where one or more amino acids are missing or have been added, respectively, at the N terminal end of the polypeptide chain. Mutated forms relate to VDRR polypeptides where one or more amino acid has been substituted by another amino acid. Suitably, the isolated or recombinant VDRR polypeptide exhibits the amino acid sequence given in Fig. 4.

The N-terminal sequence of the present nucleic acids encoding VDRR polypeptides, as well as the amino acid sequence of the present VDRR polypeptides, may vary. Thus, various N-terminal isoforms are envisaged, e.g. any of $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\gamma 1$ or $\gamma 2$ as disclosed in Fig. 7B of Transcription Factors 3: nuclear receptors, Protein Profile, vol. 2, issue 11 (1995), pp. 1173-1235. This review of nuclear receptors generally is hereby incorporated by reference. More specifically, Vitamin D receptors and related orphans, e.g. ONR1, are discussed at p. 1191-1992.

The present invention further relates to pharmaceutical formulations comprising an isolated or recombinant VDRR polypeptide, and one or more therapeutically acceptable excipients. Examples of excipients that can be used are carbohydrates, e.g. monosaccharides,

disaccharides and sugar alcohols, such as saccharose and sorbitol. Further examples include amino acids, e.g. histidine and arginine, surfactants, e.g. polyoxyethylene sorbitan fatty acid esters, inorganic salts, e.g. sodium chloride and calcium chloride, and complexing agents, e.g. EDTA and citric acid.

5 The present formulation can be in the form of an aqueous solution ready-for-use, or dried, particularly lyophilized. In the latter case, the formulation is reconstituted with a liquid, e.g. sterile water or saline, before use.

10 The present invention further relates to an expression vector comprising an isolated or recombinant nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide. The invention also relates to a cell containing such an expression vector.

The present invention further relates to a cell containing an isolated or recombinant nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide.

15 The present invention further relates to a process for recombinant production of a VDRR polypeptide, by expressing an isolated or recombinant contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide in a suitable host cell, preferably an eukaryotic cell.

20 The present invention further relates to method for identifying a ligand to a VDRR, e.g. by a cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay. It also relates to a method for identifying a substance for treatment of a condition affected by a VDRR polypeptide, comprising screening for an agonist or an antagonist of VDRR polypeptide signal transduction to be used for treating metabolic, proliferative or inflammatory conditions.

25 The present invention further relates to a VDRR polypeptide for use as a medicament, as well as use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions.

More particularly, the present invention can be used for the manufacture of medicaments for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesterolemia or hyperlipoproteinemia. The present invention can be used also for the manufacture

of medicaments for treating osteoporosis, rheumatoid arthritis, benign and malign tumors, hyperproliferative skin disorders or hyperparathyroidism.

The present invention further relates to a method for treating metabolic, proliferative or inflammatory conditions by introducing into a mammal a nucleic acid vector encoding for expression of a VDRR polypeptide. The nucleic acid vector is capable of transforming a cell *in vivo* and expressing said polypeptide in said transformed cell.

The present invention further relates to a method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR signal transduction, specifically a VDRR polypeptide.

In the present invention, the term "isolated" in connection with VDRR polypeptides or nucleic acids encoding the same, relates to nucleic acids or polypeptides that have been isolated from a natural source, e.g. the liver, small intestine or colon of a human being. The isolated VDRR polypeptides or nucleic acids of the present invention are unique in the sense that they are not found in a pure or separated form in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free environment or in a different cellular environment. The term does not imply that the sequence is the only nucleic acid or amino acid sequence present, but that it is the predominant nucleic acid or amino acid sequence present. Furthermore, the nucleic acid or polypeptide should be essentially free of non-amino acid or non-nucleic acid material naturally associated with the respective product. In this context, essentially free relates to more than 80%, suitably more than 90%, and preferably more than 95% purity.

The inventors of the present invention, have surprisingly isolated a novel nucleic acid sequence, and a polypeptide encoded by said nucleic acid sequence. Thus, a novel cDNA encoding a polypeptide designated VDRR γ has been cloned and characterized. This polypeptide is, based on amino acid sequence similarity, a novel member of the nuclear (hormone) receptor super-gene family. Hidden Markov Models (HMMs) in combination with phylogenetic analysis such as neighbor-joining tree methods and other statistical algorithms shows that VDRR γ belongs to a sub-family of vitamin D receptors (VDRs) and a VDR-like receptor from *Xenopus laevis* designated xONR1 (see Smith et al., Nucl. Acids

Res., 22 (1994), No. 1, pp. 66-71). The VD α R γ , therefore, is one member of a family of Vitamin D receptor related (VDRR) polypeptides.

This finding, in combination with the highly restricted expression pattern we observe for human VD α R γ (liver, small intestine and mucosa of colon) and in analogy to other nuclear receptors exhibiting a tissue specific expression pattern such as the peroxisome proliferator-activated receptors (PPARs) - suggest that VD α R γ performs important physiological functions in liver, small intestine and colon. Accordingly, VD α R γ is likely to be an important sensor of key metabolic pathways affecting lipid, carbohydrate or amino acid metabolism/homeostasis. In addition, the highly selective tissue specific expression pattern suggest that VD α R γ may participate in cellular differentiation and development of these tissues.

Taken together, the VDRRs in general, and more specifically the VD α R γ , may be important in

- 1) metabolic diseases such as obesity, diabetes (type I and II), lipoprotein disorders,
- 2) proliferative conditions such as tumors (benign and malignant) of the small intestine and colon,
- 3) ulcero-inflammatory diseases of small intestine and colon such as Crohn's disease and ulcerative colitis, and
- 4) congenital anomalies of small intestine and colon.

The high amino acid sequence identity of VD α R γ with the VDR both in the DNA-binding domain (DBD) and ligand-binding domain (LBD) indicate that these two receptors may also have overlapping yet distinct functional characteristics. In analogy, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) have similar amino acid sequence identities in the DBD and LBD region as the VDR and VD α R γ . RARs and RXRs have been shown to have distinct functional similarities such that both receptors bind 9-cis retinoic acid and have overlapping DNA-binding specificities and accordingly regulate overlapping gene networks. Based on these findings, VD α R γ may be regulated by small chemical molecules similar in structure to known ligands for nuclear receptors but not necessarily identical to ligands for the 1 α , 25-dihydroxy vitamin D3 receptor. Furthermore, VD α R γ may regulate vitamin D3 responsive gene networks by binding to a Vitamin D responsive element

(VDRE)-like DNA sequence. In the present application, the 1α , 25-dihydroxy vitamin D3 receptor is abbreviated as the Vitamin D receptor (VDR).

In the present invention, the substance affecting VDRR signal transduction can be any small chemical molecule of natural or synthetic origin, e.g. a carbohydrate such as an 5 aromatic compound. The small molecule may have a molecular weight in the range of from about 100 up to about 500 Da. Suitably, the small chemical molecule has a molecular weight in the range of from 200 up to 400 Da. Preferably, the small chemical molecule has a molecular weight of about 300 Da.

Genes coding for polypeptides, such as human vitamin D receptor related gamma 10 (hVDR γ), may be cloned by incorporating a DNA fragment coding for the polypeptide into a recombinant DNA vehicle, e.g. a vector, and transforming suitable prokaryotic or eukaryotic host cells. Such recombinant DNA techniques are well known and e.g. described in Methods in Enzymology, Academic Press, San Diego, CA, USA (1994), vols. 65 and 68 (1979), and vols. 100 and 101 (1983).

15 The host cells for use in the present invention can be prokaryotic or eukaryotic, preferably eukaryotic cells. Suitable eukaryotic host cells include but are not limited to cells from yeast, e.g. Saccharomyces, insect cells and mammalian cells such as Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK), COS and the like. Suitable prokaryotic host cells include but are not limited to cells from Enterobacteriaceae, e.g. E. coli, Bacillus and 20 Streptomyces.

EXAMPLES

The following Examples are provided for purposes of illustration only and are not to 25 be construed as in any way limiting the scope of the present invention, which is defined by the appended claims.

EXAMPLE 1Identification and isolation of human VDRR γ cDNA

Expressed Sequence Tag (EST) databases were screened for nuclear receptor related sequences with a DNA-binding domain (DBD) profile of nuclear receptors. This search profile was created by multiple alignment of a selected set of nuclear receptor sub-domains followed by a statistical calculation to obtain a so called Hidden Markov Model (HMM) of different subfamily members of the nuclear receptor supergene family. The cDNA of one of the nuclear receptor related EST sequences identified (Incyte clone no 2211526) was analyzed in detail by sequencing. After DNA sequencing of the entire Incyte cDNA clone (approximately 2200 basepairs) the clone was found to encode a putative ligand-binding domain (LBD) with 54% and 44% similarity to xONR-1 and to the vitamin D receptor (VDR), respectively. The cDNA of the Incyte clone was not full-length and did not encode a sequence corresponding to a complete DBD.

5'-RACE (rapid amplification of cDNA ends) of random primed cDNA from human liver RNA (InVitrogen) followed by cloning and DNA sequencing showed that the 5'-part of the cDNA corresponding to the Incyte clone encoded a DBD characteristic for nuclear receptors and with 71% and 65% sequence identity to xONR-1 and VDR, respectively. Multiple alignments in combination with evolutionary neighbor-joining tree analysis placed the polypeptide encoded by the cDNA (specified in Fig. 1) in the group of VDRs (Figs. 2 10 and 3) and was named human vitamin D receptor related gamma (VDRR γ). The deduced amino acid sequence of VDRR γ is given in Fig. 4.

EXAMPLE 2Expression of VDRR γ mRNA in human tissues

Multiple tissue northern blots (Clontech) was used to determine the expression pattern of VDRR γ in adult human tissues. As shown in Fig. 5, VDRR γ is abundantly expressed in small intestine, mucosal lining of colon and liver but not in several other tissues including spleen, thymus, prostate, testis, ovary, peripheral blood leukocytes, heart, brain, placenta, lung, skeletal muscle, kidney and pancreas. The restricted expression pattern of VDRR γ suggest that this receptor is likely to have an important regulatory function in liver and intestine.

EXAMPLE 3Transient transfections of GAL4-DBD/VDRR γ -LBD fusion protein using Vitamin D3

- 5 Transient transfections were performed to analyze if vitamin D3 activate the VDR γ polypeptide. To this end, transient co-transfections of CV-1 cells were performed with expression plasmids encoding fusion proteins of the GAL4-DBD fused to the LBD of either the VDR or the VDR γ together with a reporter-plasmid containing five GAL4 responsive elements upstream of the luciferase gene. After transfection, cells were treated with vehicle
- 10 (DMSO) alone or with vitamin D3 for 48 hours followed by harvesting of the cells and measurement of the luciferase activity in cell extracts. As shown in Fig. 6, vitamin D3 (1 μ M) transactivate the GAL4-DBD/VDR-LBD but not the corresponding GAL4-DBD/-VDR γ -LBD polypeptide under these conditions. This indicates that the two receptors may have distinct ligand-binding specificities.

CLAIMS

1. An isolated or recombinant nucleic acid comprising a contiguous nucleic acid sequence
5 encoding a vitamin D receptor related (VDRR) polypeptide.
2. The nucleic acid according to claim 1, wherein said VDRR polypeptide is of mammalian,
preferably human, origin.
- 10 3. The nucleic acid according to claims 1 or 2, encoding the VDRR polypeptide containing a
DNA-binding domain (DBD) comprising about 77 amino acids with 9 cysteine residues,
wherein said DBD is further characterized by the following amino acid sequence identity
relative to the DBDs of human Vitamin D Receptor (hVDR) and Orphan Nuclear Receptor 1
isolated from *Xenopus laevis* (xONR1), respectively:
 - 15 (i) at least about 50% amino acid sequence identity with the DBD of hVDR; and
 - (ii) at least about 55% amino acid sequence identity with the DBD of xONR1.
4. The nucleic acid according to claim 3, wherein said DBD is characterized by the
following amino acid sequence identity:
- 20 (i) at least 60% amino acid sequence identity with the DBD of hVDR; and
- (ii) at least 65% amino acid sequence identity with the DBD of xONR1.
5. The nucleic acid according to claim 4, wherein said DBD is characterized by the
following amino acid sequence identity:
- 25 (i) about 65% amino acid sequence identity with the DBD of hVDR; and
- (ii) about 71% amino acid sequence identity with the DBD of xONR1.
6. The nucleic acid according to any previous claim, encoding the VDRR polypeptide,
wherein the ligand-binding domain (LBD) of said polypeptide is characterized by the
30 following amino acid sequence identity, relative to the LBDs of hVDR and xONR1,
respectively:

- (i) at least about 30% amino acid sequence identity with the LBD of hVDR; and
- (ii) at least about 40% amino acid sequence identity with the LBD of xONR1.

7. The nucleic acid according to claim 6, wherein said LBD is characterized by the following

5 amino acid sequence identity:

- (i) at least 35% amino acid sequence identity with the LBD of hVDR; and
- (ii) at least 45% amino acid sequence identity with the LBD of xONR1.

8. The nucleic acid according to claim 7, wherein said LBD is characterized by the following

10 amino acid sequence identity:

- (i) about 42% amino acid sequence identity with the LBD of hVDR; and
- (ii) about 54% amino acid sequence identity with the LBD of xONR1.

9. The nucleic acid according to any previous claim, wherein said nucleic acid sequence is

15 that given in Fig. 1.

10. A nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR polypeptide in a sample.

20 11. The nucleic acid probe according to claim 10, wherein said probe comprises at least 14 contiguous nucleotides of the nucleic acid sequence given in Fig. 1.

12. A method for identifying clones encoding a VDRR polypeptide said method comprising screening a genomic or cDNA library with a nucleic acid probe according to claims 10 or 11 under low stringency hybridization conditions, and identifying those clones which display a substantial degree of hybridization to said probe.

25 13. An expression vector comprising a nucleic acid according to claim 1.

30 14. A cell containing a nucleic acid according to claim 1.

15. A cell containing an expression vector according to claim 13.
16. A process for recombinant production of a VDRR polypeptide, said process comprising expressing the nucleic acid of claim 1 in a suitable host cell.
- 5 17. The process according to claim 16, wherein the host cell is eukaryotic.
18. An isolated or recombinant VDRR polypeptide.
- 10 19. The isolated or recombinant VDRR polypeptide according to claim 18 comprising the amino acid sequence given in Fig. 4.
- 15 20. A pharmaceutical formulation comprising an isolated or recombinant VDRR polypeptide according to claims 18 or 19, and one or more therapeutically acceptable excipients.
21. A method for identifying a ligand to a VDRR, by a cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay.
- 20 22. A method for identifying a substance for treatment of a condition affected by a VDRR polypeptide, comprising screening for an agonist or an antagonist of VDRR polypeptide signal transduction to be used for treating metabolic, proliferative or inflammatory conditions.
- 25 23. A VDRR polypeptide for use as a medicament.
24. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions.

25. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesterolemia or hyperlipoproteinemia.

5 26. Use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating osteoporosis, rheumatoid arthritis, benign and malign tumors, hyperproliferative skin disorders or hyperparathyroidism.

10 27. Use according to any of claims 24-26, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.

15 28. A method for treating metabolic, proliferative or inflammatory conditions comprising introducing into a mammal a nucleic acid vector according to claim 13 encoding for expression of a VDRR polypeptide and wherein said nucleic acid vector is capable of transforming a cell *in vivo* and expressing said polypeptide in said transformed cell.

20 29. A method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR signal transduction.

25 30. The method according to claim 29, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.

ABSTRACT

The present invention relates to novel vitamin D receptor related (VDRR) poly-peptides, and formulations containing the same. Nucleic acid sequences encoding the VDRR polypeptides, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention. The invention further relates to VDRR polypeptides for use as medicaments, and use of substances affecting VDRR signal transduction for the manufacture of medicaments for treating metabolic, proliferative or inflammatory conditions. The present invention also relates to methods for identifying clones encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods for identifying substances for treatment of conditions affected by a VDRR polypeptide. More specifically, the novel VDRR polypeptide can be the polypeptide designated VDR γ , which may be regulated by any small chemical molecule similar in structure to known ligands for nuclear receptors.

1 CCTCTGAAGG TTCTAGAAC GATAGTGAAT TCGTGGGACG GGAAGAGGAA
51 GCACTGCCTT TACTCAGTG GGAATCTCGG CCTCAGCCTG CAAGCCAAGT
101 GTTCACAGTG AAAAAAGCAA GAGAATAAGC TAATACTCCT GTCCTGAACA
151 AGGCAGCGGC TCCTTGGTAA AGCTACTCCT TGATCGATCC TTTGCACCGG
201 ATTGTTAAA GTGGACCCCA GGGGAGAAGT CGGAGCAAAG AACTTACAC
251 CAAGCAGTCC AAGAGGCCCA GAAGCAAACC TGGAGGTGAG ACCCAAAGAA
301 AGCTGGAACC ATGCTGACTT TGTACACTGT GAGGACACAG AGTCTGTTCC
351 TGGAAAGCCC AGTGTCAACG CAGATGAGGA AGTCGGAGGT CCCCAAATCT
401 GCCGTGTATG TGGGGACAAG GCCACTGGCT ATCACTTCAA TGTATGACA
451 TGTGAAGGAT GCAAGGGCTT TTTCAGGAGG GCCATGAAAC GCAACGCCG
501 GCTGAGGTGC CCCTTCCCGA AGGGCGCCTG CGAGATCACC CGGAAGACCC
551 GGCGACAGTG CCAGGCCTGC CGCCTGCGCA AGTGCCTGGA GAGCGGCATG
601 AAGAAGGAGA TGATCATGTC CGACGAGGCC GTGGAGGAGA GGCAGGGCCTT
651 GATCAAGCGG AAGAAAAGTG AACGGACAGG GACTCAGCCA CTGGGAGTGC
701 AGGGGCTGAC AGAGGAGCAG CGGATGATGA TCAGGGAGCT GATGGACGCT
751 CAGATGAAAA CCTTGACAC TACCTTCTCC CATTCAAGA ATTTCCGGCT
801 GCCAGGGGTG CTTAGCAGTG GCTGCGAGTT GCCAGAGTCT CTGCAGGCC
851 CATCGAGGGA AGAAGCTGCC AAGTGGAGCC AGGTCCGGAA AGATCTGTGC
901 TCTTGAAAGG TCTCTTGCA GCTGCGGGGG GAGGATGGCA GTGTCTGGAA
951 CTACAAACCC CCAGCCGACA GTGGCGGGAA AGAGATCTTC TCCCTGCTGC
1001 CCCACATGGC TGACATGTCA ACCTACATGT TCAAAGGCAT CATCAGCTTT
1051 GCCAAAGTCA TCTCCTACTT CAGGGACTTG CCCATCGAGG ACCAGATCTC
1101 CCTGCTGAAG GGGGCCGCTT TCGAGCTGTG TCAACTGAGA TTCAACACAG
1151 TGTTCAACGC GGAGACTGGA ACCTGGGAGT GTGCCGGCT GTCCTACTGC
1201 TTGGAAGACA CTGCAGGTGG CTTCCAGCAA CTTCTACTGG AGCCCATGCT
1251 GAAATTCCAC TACATGCTGA AGAAGCTGCA GCTGCATGAG GAGGAGTATG
1301 TGCTGATGCA GGCCATCTCC CTCTTCTCCC CAGACCGCCC AGGTGTGCTG
1351 CAGCACCGCG TGGTGGACCA GCTGCAGGAG CAATTGCCA TTACTCTGAA
1401 GTCCTACATT GAATGCAATC GGCCCCAGCC TGCTCATAGG TTCTTGTCC

Fig. 1

1451 TGAAGATCAT GGCTATGCTC ACCGAGCTCC GCAGCATCAA TGCTCAGCAC
1501 ACCCAGCGGC TGCTGCGCAT CCAGGACATA CACCCCTTG CTACGCCCT
1551 CATGCAGGAG TTGTTGGCA TCACAGGTAG CTGAGCGGCT GCCCTGGGT
1601 GACACCTCCG AGAGGCAGCC AGACCCAGAG CCCTCTGAGC CGCCACTCCC
1651 GGGCCAAGAC AGATGGACAC TGCCAAGAGC CGACAATGCC CTGCTGGCCT
1701 GTCTCCCTAG GGAATTCTG CTATGACAGC TGGCTAGCAT TCCTCAGGAA
1751 GGACATGGGT GCCCCCCACC CCCAGTCAG TCTGTAGGGA GTGAAGCCAC
1801 AGACTCTTAC GTGGAGAGTG CACTGACCTG TAGGTCAGGA CCATCAGAGA
1851 GGCAAGGTTG CCCTTCCTT TTAAAAGGCC CTGTGGTCTG GGGAGAAATC
1901 CCTCAGATCC CACTAAAGTG TCAAGGTGTG GAAGGGACCA AGCGACCAAG
1951 GATAGGCCAT CTGGGGTCTA TGCCCACATA CCCACGTTG TTCGCTTCCT
2001 GAGTCTTTC ATTGCTACCT CTAATAGTCC TGTCTCCAC TTCCCACTCG
2051 TTCCCCCTCCT CTTCCGAGCT GCTTGTGGG CTCAAGGCCT GTACTCATCG
2101 GCAGGTGCAT GAGTATCTGT GGGAGTCCTC TAGAGAGATG AGAACCCAGG
2151 AGGCCTGCAC CAAATGTCAG AAGCTTGGCA TGACCTCATT CGGCCACAT
2201 CATTCTGTGT CTCTGCATCC ATTGAACAC ATTATTAAGC ACTGATAATA
2251 GGTAGCCTGC TGTGGGTAT ACAGCATTGA CTCAGATATA GATCCTGAGC
2301 TCACAGAGTT TATAGTTAAA AAAACAAACA GAAACACAAA CAATTGGAT
2351 CAAAAGGAGA AAATGATAAG TGACAAAAGC AGCACAAGGA ATTTCCCTGT
2401 GTGGATGCTG AGCTGTGATG GCAGGCAC TGTAACCAAG TGAAGGTTCC
2451 CGAGGACATG AGTCTGTAGG AGCAAGGGCA CAAACTGCAG CTGTGAGTGC
2501 GTGTGTGTGA TTTGGGTAG GTAGGTCTGT TTGCCACTTG ATGGGGCCTG
2551 GGTTTGTCC TGGGGCTGGA ATGCTGGTA TGCTCTGTGA CAAGGCTACG
2601 CTGACAATCA GTTAAACACA CCGGAGAAGA ACCATTTACA TGCACCTTAT
2651 ATTTCTGTGT ACACATCTAT TCTCAAAGCT AAAGGGTATG AAAGTGCCTG
2701 CCTTGTAT AGCCACTTGT GAGTAAAAT TTTTTGCAT TTTCACAAAT
2751 TATACTTTAT ATAAGGCATT CCACACCTAA GAACTAGTTT TGGGAAATGT
2801 AGCCCTGGGT TTAATGTCAA ATCAAGGCAA AAGGAATTAA ATAATGTACT
2851 TTTGGCTAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
2901 AAAAAA

Fig. 1 (cont.)

Evolutionary Neighbour-Joining Tree

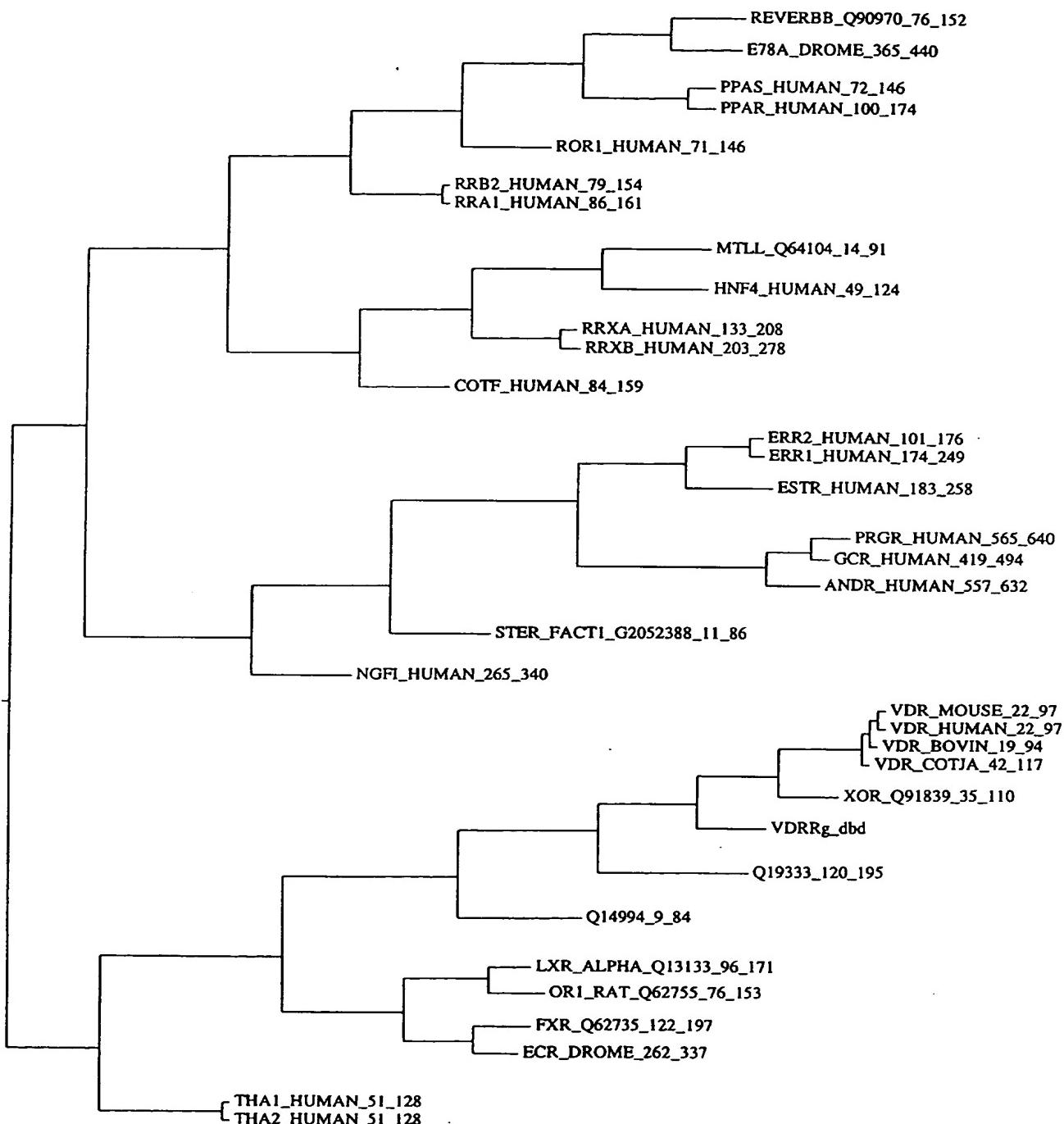


Fig. 2

Evolutionary Neighbour-Joining Tree

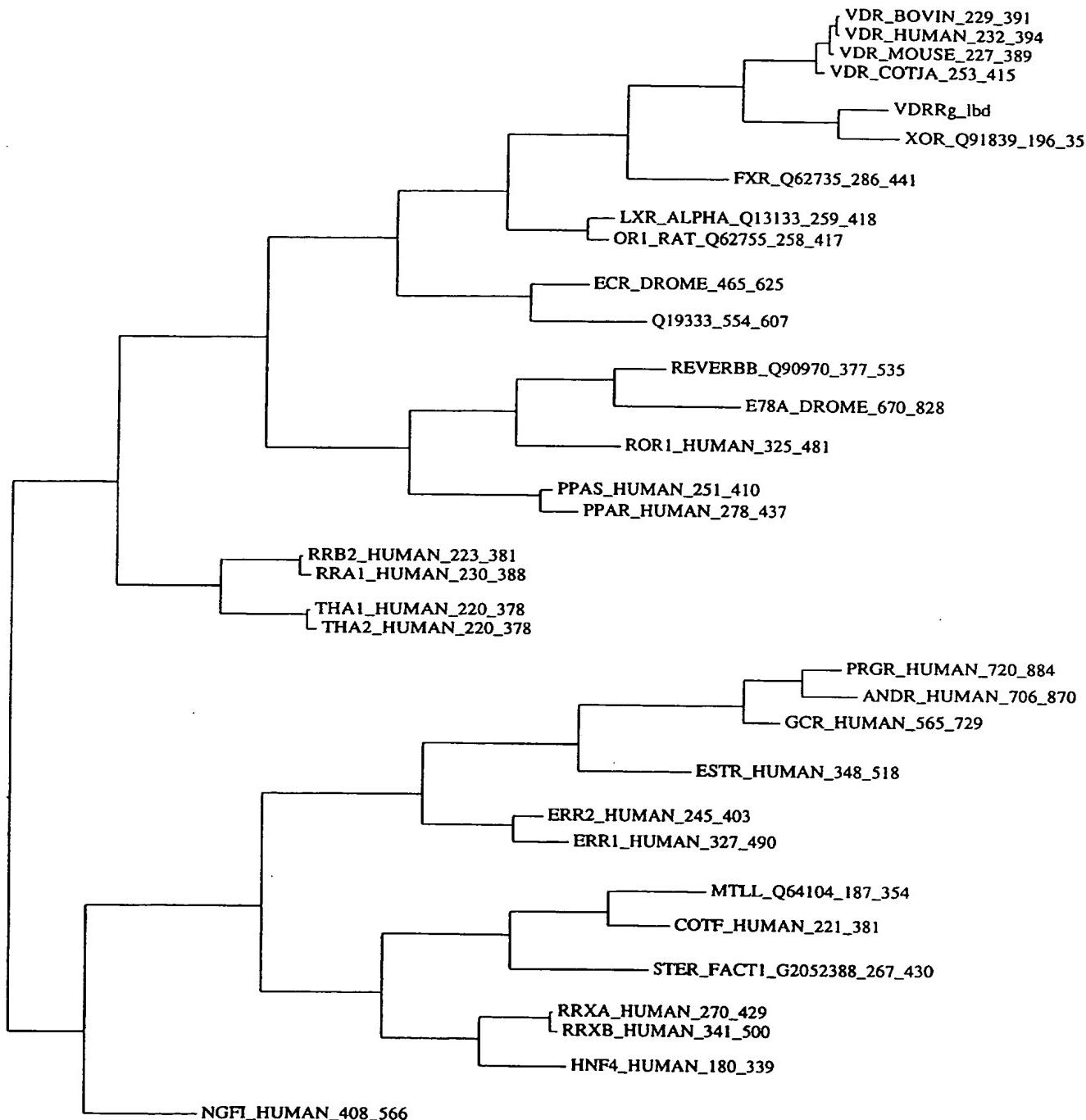


Fig. 3

5/7

1 MEVRPKESWN HADFVHCEDT ESVPGKPSVN ADEEVGGPQI CRVCGDKATG

51 YHFNVMTCEG CKGFFRRAMK RNARLRCPFR KGACEITRKT RRQCQACRLR

101 KCLESGMKKE MIMSDEAVEE RRALIKRKKS ERTGTQPLGV QGLTEEQRMM

151 IRELMDAQMK TFDTTFSHK NFRLPGVLSS GCELPESLQA PSREEAAKWS

201 QVRKDLCQLK VSLQLRGEDG SVWNYKPPAD SGGKEIFSLL PHMADMSTYM

251 FKGIIISFAKV ISYFRDLPPIE DQISLLKGAA FELCQLRFNT VFNAETGTWE

301 CGRLSYCLED TAGGFQQQLL EPMLKFHYML KKLQLHEEEY VLMQAISLFS

351 PDRPGVLQHR VVDQLQEQQFA ITLKSYIECN RPQPAHRFLF LKIMAMLTEL

401 RSINAQHTQR LLRIQDIHPF ATPLMQELFG ITGS

Fig. 4

6/7

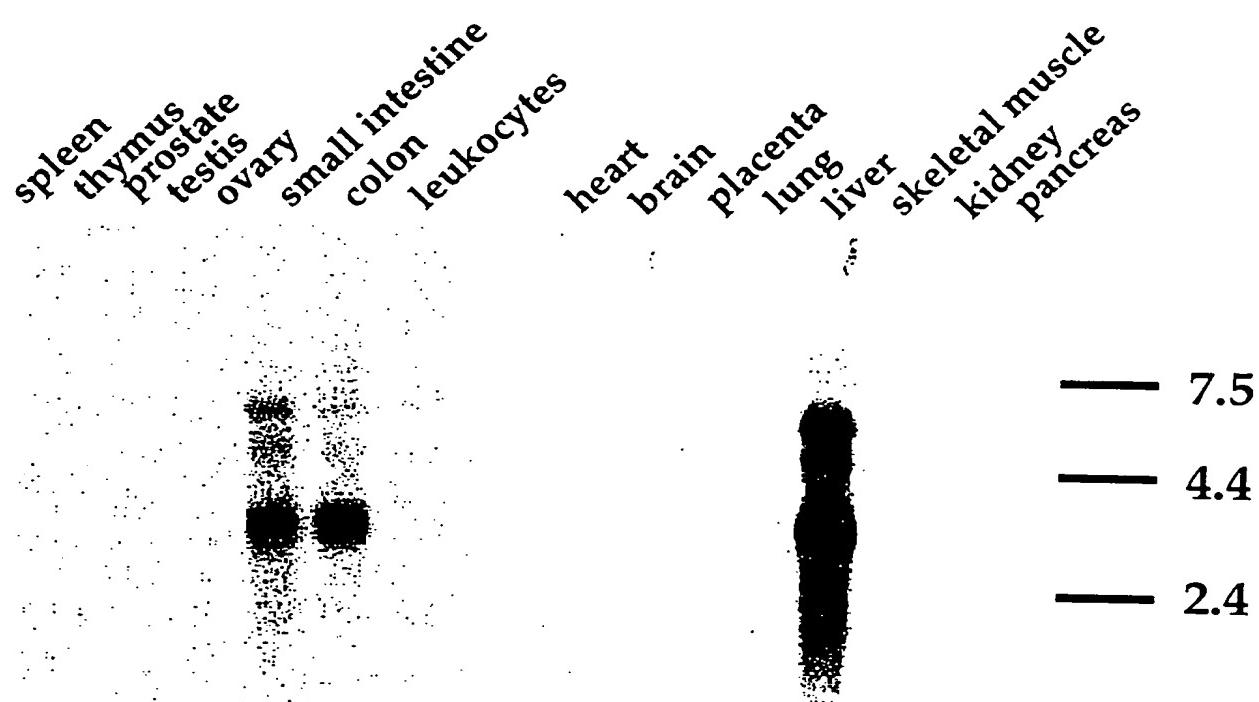


Fig. 5

7/7

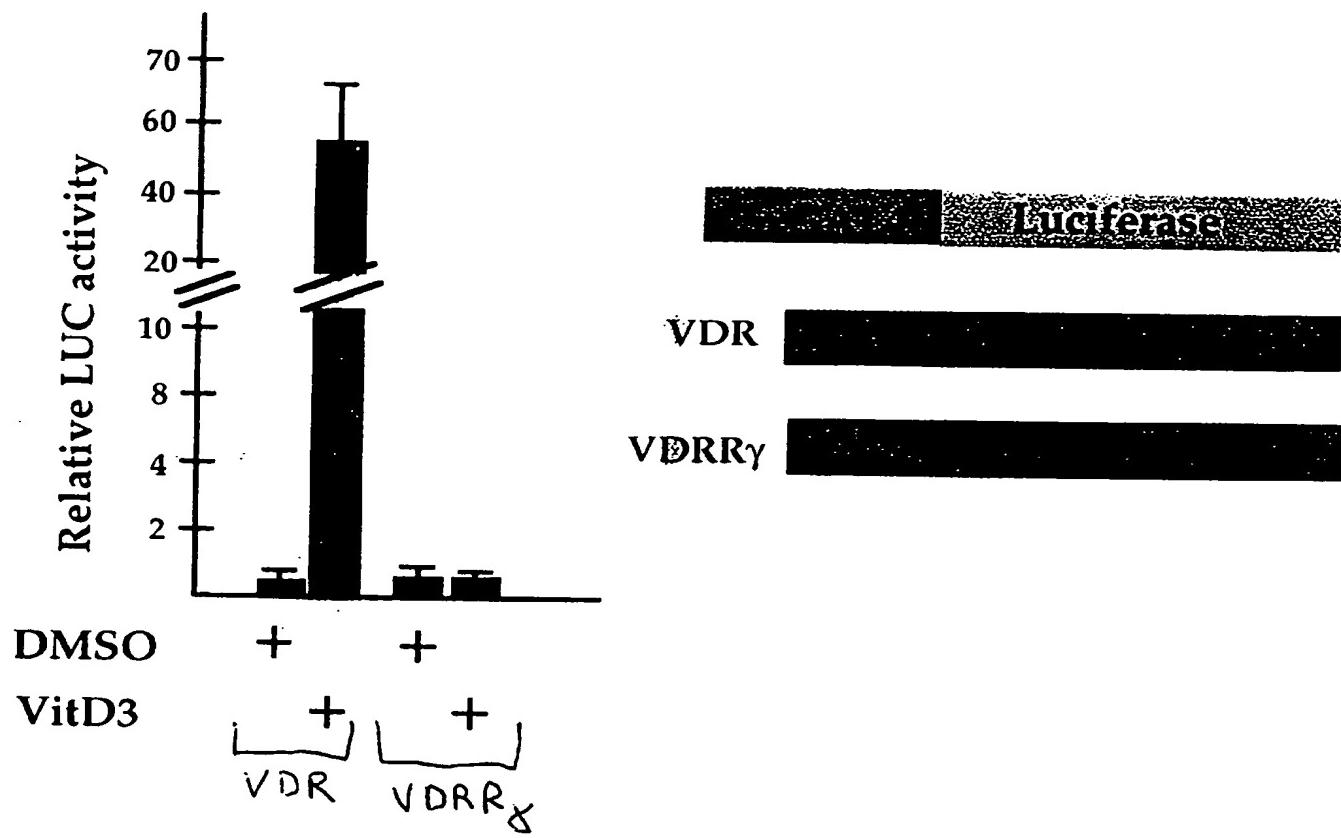


Fig. 6